In search of animal models for male sexual dysfunction

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Chapter 6

Pharmacological studies on the role of 5-HT1A receptors in male sexual behavior of wildtype and serotonin transporter knockout rats

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Abstract

Background: Brain serotonin (5-HT) neurotransmission plays an important role in male sexual behavior and it is well established that activating 5-HT_{1A} receptors in rats facilitates ejaculatory behavior. However, the relative contribution of somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} heteroreceptors in this pro-sexual behavior is unclear.

Objective: To determine the role of presynaptic auto-receptors versus postsynaptic heteroreceptors in the pro-sexual effects of 5-HT_{1A} receptor activation.

Experimental approach: The acute effects of the biased 5-HT_{1A} receptor agonists F-13714 (0-0.16 mg/kg, IP), a preferential 5-HT_{1A} autoreceptor agonist, or F-15599 (0-0.64 mg/kg, IP), a preferential 5-HT_{1A} heteroreceptor agonist, andS15535 (0-4 mg/kg, IP) a mixed 5-HT_{1A} autoreceptor agonist/heteroreceptor antagonist, on male sexual behavior were assessed in wildtype and serotonin transporter knockout ($SERT^{-/-}$) Wistar rats. The latter animals, exhibiting enhanced extracellular 5-HT levels and desensitized 5-HT_{1A} receptors, model neurochemical changes underlying chronic SSRI-induced sexual dysfunction.

Key results: A clear and stable genotype effect was found after training where $SERT^{+/+}$ male rats performed sexual behavior at a higher level than $SERT^{-/-}$. Both F-15599 and F-13714 induced pro-sexual activity in $SERT^{+/+}$ and $SERT^{-/-}$ animals. Compared to $SERT^{+/+}$ rats, the F13714- dose-response curve in $SERT^{-/-}$ rats was, shifted to the right. $SERT^{+/+}$ and $SERT^{-/-}$ rats responded similar to F15599. Within both $SERT^{+/+}$ and $SERT^{-/-}$ rats the potency of F-13714 was much stronger compared to F-15599. S15535 had no effect on sexual behavior in either genotype.

Conclusions: The two biased compounds with differential effects on 5-HT_{1A} auto- and hetero-receptors, exerted pro-sexual activity in both $SERT^{+/+}$ and $SERT^{-/-}$ rats. This makes interpretations whether pre-or postsynaptic 5-HT_{1A} receptors are involved in prosexual activity rather difficult. Moreover, the inactivity of S15535 in sexual behavior in either genotype was unexpected and difficult to explain. The question remains whether the in vivo activity profile of the various 5-HT_{1A} receptor ligands used, is sufficient to differentiate possible contributions of pre-and/or postsynaptic 5-HT_{1A}R in male rat sexual behavior.

Keywords: serotonin, male sexual behavior, rat, 5-HT_{1A} receptor, serotonin transporter, 5-HT_{1A} autoreceptors, 5-HT_{1A} heteroreceptors.

1.Introduction

The serotonergic system plays an important modulatory role in sexual behavior (Uphouse & Guptarak, 2010). This is, for example, illustrated by the effects of chronic SSRI treatment in depressed patients that results in enhanced 5-HT levels often causing sexual dysfunctions like in men delayed ejaculation and libido problems (Segraves & Balon, 2014). Early studies in male rats identified 5-HT_{1A} receptor (R) agonists like 8-OH-DPAT, the azapirones (e.g. buspirone, ipsapirone and gepirone) and others (e.g. flesinoxan) as pro-sexual drugs (Ahlenius et al., 1981; Ahlenius & Larsson, 1985; reviewed in: Snoeren et al., 2014). The prototypal 5-HT_{1A} R agonist ((+/-) and (+) - 8-OH-DPAT, potently stimulates male rat sexual behavior; in a certain time frame (e.g. 30 min), the number of ejaculations increases associated with shortened ejaculation latencies and fewer intromissions to reach ejaculation (Chan et al., 2011; Hillegaart & Ahlenius, 1998; Uphouse & Guptarak, 2010).

5-HT_{1A} receptors are present as presynaptic inhibitory autoreceptors on soma and dendrites of raphé serotonergic neurons projecting to many forebrain areas (Altieri et al., 2013; Fernandez-Guasti et al., 1992; Le Poul et al., 1995; Marek, 2010). Moreover, 5-HT_{1A} receptors are also present as postsynaptic heteroreceptors in various brain areas, mainly in the forebrain (Frink et al., 1996; Garcia-Garcia et al., 2017). Systemic acute administration of non-selective 5-HT_{1A} R agonists (activation of pre- and postsynaptic receptors) leads to decreased serotonergic release, but at the same time to activation of postsynaptic 5-HT_{1A} heteroreceptors (Lladó-Pelfort et al, 2012; Müller et al., 2007). The resulting behavioral outcome (facilitation of male sexual behavior) is rather difficult to explain by this complex mechanism underlying activation of all 5-HT_{1A} receptors.

To further explore the role of pre- and postsynaptic 5-HT_{1A} receptors in male sexual behavior, more recently developed selective and high-affinity 5-HT_{1A}R agonists are useful. These so-called 'biased' or 'functionally selective' agonists (Garcia-Garcia et al., 2014; Newman-Tancredi, 2011) display selectivity for either pre- or postsynaptic 5-HT_{1A} receptors. F15599 is a high-affinity, selective 5-HT_{1A} R agonist (Ki=3.4 nM) for postsynaptic 5-HT_{1A} heteroreceptors, whereas F13714 (Ki=0.1nM) is a preferential 5-HT_{1A} autoreceptor agonist (Koek et al., 2001; De Boer & Newman-Tancredi, 2016; Hazari et al., 2017). We studied both compounds in a dose-response study in male rat sexual behavior. Another high-affinity (Ki=1.8 nM) 5-HT_{1A} R ligand, S-15535 acts in vivo as a preferential agonist at presynaptic autoreceptors and as antagonist at postsynaptic 5-HT_{1A} heteroreceptors (Carli et al., 1999; Millan et al., 1993).This compound is an interesting tool to study in male sexual behavior as it may shed further light on the complex role of 5-HT_{1A} receptors in male rat sexual behavior.

Male rats lacking the serotonin transporter ($SERT^{-/-}$) consist of a robust genotype that have a lower basal ejaculatory performance than wildtype rats ($SERT^{+/+}$) or heterozygous serotonin transporter knockout ($SERT^{+/-}$) rats (Chan et al., 2011a; Esquivel-Franco et al., 2018). This genetic animal model has also been proposed and used as an animal model of spontaneous or SSRI-induced sexual dysfunction in men, which is believed to be caused by the combination of enhanced 5-HT levels and diminished 5-HT_{1A} receptor functioning similar to chronic SSRI-treatment in normal animals (Chan et al., 2011). In particular this 5-HT_{1A} R desensitization phenomenon is relevant here to further provide more clarity as to the potency of the biased agonists to stimulate sexual behavior. $SERT^{-/-}$ rats have higher extracellular serotonin levels than $SERT^{+/+}$ animals which is comparable to chronic SSRI administration (Homberg et al., 2007). Pharmacological experiments in these rats indicated that rats lacking the SERT have altered 5-HT_{1A} receptor reactivity; the altered 5-HT_{1A} receptor functioning is probably not a global phenomenon, but might be limited to some specific subpopulations of 5-HT_{1A} receptors, as indicated by changed autonomic responses like core body temperature in $SERT^{+/+}$ and $SERT^{-/-}$ animals (Homberg et al., 2008; Olivier et al., 2008), experiments performed in male sexual behavior (Chan et al. 2011) also indicated that at least two populations of 5-HT_{1A} receptors are involved in its expression. For normal sexual behavior, activation of one population of 5-HT_{1A} receptors is needed and this pool is desensitized in $SERT^{-/-}$ rats. The pro-sexual effects of 8-OH-DPAT are mediated via 5-HT_{1A} receptors, which are less sensitive in $SERT^{-/-}$ rats. This difference makes the $SERT^{-/-}$ rat a further attractive model to test the different 5-HT_{1A} receptor-modulating drugs, F15599, F13714 and S-15355. Finally, we selected male rats that, after extensive training (Pattij et al., 2005), display a low level of sexual behavior, i.e. ejaculations. Because 5-HT_{1A} receptor agonists facilitate ejaculation, a too high initial level of the number of ejaculations would probably interact with the pro-sexual effects of these drugs. To identify the role of somatodendritic (auto)receptors and post (hetero) receptors we used F13714, F15599 and S15535 in $SERT^{+/+}$ and $SERT^{-/-}$ rats.

2. Materials and methods

2.1 Animals

Wistar rats were bred in our animal facility (University of Groningen, GELIFES) using serotonin transporter (SERT) heterozygous males and females, resulting in male and female SERT wild type ($SERT^{+/+}$), heterozygous ($SERT^{+/-}$) and homozygous or knock out ($SERT^{-/-}$) rats. We used two batches of animals, the first one consisting of sixty-three male SERT ($SERT^{+/+}$, n=32), and ($SERT^{-/-}$, n=31) rats and the second one of thirty-two male (16 $SERT^{+/+}$ and 16 SERT) rats, all of them of at least 12 weeks old when used for sexual behavior experiments.

Female $SERT^{+/-}$ and $SERT^{+/+}$ were used as sexual stimulus females (n=120). Rats were housed under reversed dark-light conditions (12h light:12h dark, lights off from 8:00 AM to 8 PM). After 6-weekly training tests (30 min/test), male rats were considered sexually trained and classified based on ejaculation frequencies per test. Male rats display, after extensive training, a rather stable sexual phenotype (Chan et al., 2008; Olivier et al., 2006; Pattij et al., 2005). In these experiments, for batch one we selected rats that showed a normal ejaculatory phenotype (between 1 and 2 ejaculations per test after training, for the last three sessions) and for the second batch rats that showed a rather low sexual phenotype (between 0 and 1 ejaculation per test after training, for the last three sessions). Animals were socially housed (2-5 per cage, maximum 4 for males). Cages were enriched with wooden gnawing blocks and nesting material (Envirodri). A total of ninety-five males (batch one and two) were sexually trained for 6 weeks and a total of 45 male rats (batch one + batch two) with a normal and low average number of ejaculations, respectively were selected (because this enhances the sensitivity of the anticipated improvement in sexual behavior by the $5\text{-}HT_{1A}$ compounds and to match the control group as much as possible to the knock-out animals). All the experiments, lasted 13 weeks in total (after training). In all individual experiments of batch one 12 rats per genotype ($SERT^{+/+}$ and $SERT^{-/-}$) were used, and for batch two we used 10 $SERT^{+/+}$ and 11 $SERT^{-/-}$ rats; animals were used only once a week to guarantee sufficient drug washout time. Rats had *ad libitum* access to food and water. All experiments were conducted in accordance with the governmental guidelines for care and use of laboratory animals (Centrale Commissie Dierproeven) and were approved by the Ethical Committee for Animal Research of the Groningen Institute for Evolutionary Life Sciences at University of Groningen. All efforts were made to minimize the number of animals and their suffering.

2.2 Female rats

The female stimulus rats were tubal ligated in order to prevent pregnancies. To perform tubal ligation surgery, females were anaesthetized (Isoflurane) and given pain relief (Fynadine, 0.1mg/100g) before the surgery, and 24 and 48 hours after surgery. Females were at least 12 weeks old when surgery was performed, and two weeks of recovery were given before they were made intentionally receptive with estradiol (50 µg in 0.1 ml oil, S.C., 36-48 hours before the test) for the sexual behavior training tests and experiments. Females were used not more than once in two weeks and not more than 2 times per experimental day.

2.3 Drug treatment and behavioral experiments

For the first batch, animals received all dosages of F13714 and F15599 in a crossover-randomized design in order to prevent order effects; after this experiment, S15535 was administered in a randomized design. For the second batch, animals were only administered S15535 in a randomized design like the first set of animals. As described previously in Olivier et al. (2017), when pharmacological tests were performed, male rats were given a 30-min habituation time in the test boxes right after drug administration via IP injection, before the female rat was introduced. All behavior during the 30-minute test was video-recorded after introduction of the female and were also live-scored, and the following parameters of the ejaculation series were deduced (Chan et al., 2011): number of ejaculations/test (E), number of mounts (M), number of intromissions (I), latency (s) to first mount (ML), latency (s) to first intromission (IL) and latency (s) to the first ejaculation (EL). After ejaculation, the post ejaculatory latency (PEL(s),) was calculated, using the time from the first ejaculation and the time of the first mount/intromission (whatever occurred first) of the second ejaculation series. Intromission Ratio (IR) was calculated as: IR = *(#I/ (#I + #M)) *100%*. EL was calculated using the time of the EL from the first ejaculation series minus the intromission latency of the first ejaculation series (EL= EL - IL). These parameters were used to run the statistical analysis.

Because it is important to have comparable pharmacodynamics and kinetics in pharmacological studies, a test of fixed duration has been chosen; 30 minutes (1800 sec). In the cases where drug-treatment had no "effect" on ejaculation and sexual behavior, or few or no animal achieved a first ejaculation it was not possible to perform statistical analyses and therefore, for those cases, we assigned values of 1800 sec (i.e. the maximum test duration) for some latencies (ejaculation, mount and intromission latency), although this is undoubtedly a matter of discussion as we have mentioned before (Chan et al., 2011b; Olivier et al., 2017). All tables and figures show the results for the first Ejaculation Series.

2.4 Drugs

F15599 and F13714 (Pierre Fabre Pharmaceuticals, France; Lot # SBR1401003 and # JLM3001201, resp.) and S-15535 (Servier Pharmaceuticals, France; Lot B01JLP061A) were dissolved in NaCl 0.9% (saline) and each solution was freshly prepared on each testing day. All drugs were administered via intraperitoneal (IP) injection 30 minutes before the test.

2.5 Training

For the first batch, $SERT^{+/+}$ (N=32) and $SERT^{-/-}$ (N=31) male rats were sexually trained 6 times (30 min, once a week); and for the second batch $SERT^{+/+}$ (N=16) and $SERT^{-/-}$ (N=16) male rats were sexually trained 10 times (30 min, once a week, they received extra training due to the extreme low sexual performance) to assess and stabilize their basal sexual activity. Rats had a habituation period of 10 minutes in the testing box right before the training session. At the end of the habituation period a receptive female was introduced in the box and sexual behavior was assessed for 30 minutes. Non-receptive females were switched for a different receptive female. The training and testing occurred in wooden rectangular (57 cm x 82 cm x 39 cm; glass wall) testing boxes filled with regular bedding material. To stimulate sexual behavior, bedding material was not changed during the training and testing to preserve pheromones of previous rounds and to create a more competitive sexual environment. Only males showing stable normal (1-2 ejaculations, for batch 1) and low (0-1 ejaculations for batch 2) ejaculation levels on the last three tests, 24 in total were used for the pharmacological experiments (batch one: N=12 per genotype); batch two in total 21 (10 $SERT^{+/+}$ and11 $SERT^{-/-}$ rats). All training sessions and experiments were performed under red light conditions between 10:00 AM and 17:00 PM

2.6 Pharmacological Experiments

Batch 1

Experiment one: F15599 and F13714 dose response. 24 normal ejaculating male rats were selected (N=12 per SERT genotype) and went on a crossover design. Rats received vehicle (saline); 0.01, 0.04, 0.16 and 0.64-mg/kg F15599 and 0.0025, 0.01, 0.04 and 0.16-mg/kg F13714 via IP. Experiments were performed once per week on the same testing day, over nine weeks and animals and treatment were randomized over the nine weeks. Although the experiments with these two drugs were performed together, we decided to perform the statistical analysis separately for each compound.

Experiment two: S15535 dose response. The same 24 animals from experiment one received vehicle (saline), 0.25, 1 and 4-mg/kg S15535, IP in a randomized design. Testing was performed over four weeks and always on the same day per week.

Batch 2

Experiment two: S15535 dose response. 10 $SERT^{+/+}$ and 11 $SERT^{-/-}$ rats were selected for low number of ejaculations. Rats received vehicle (saline), 0.25, 1 and 4-mg/kg S15535, IP in a randomized design. Testing was performed over four weeks and always on the same day per week.

2.7 Statistical analyses

Differences in baseline ejaculation numbers during the training between genotypes were analyzed using two-way ANOVA for repeated measures, with genotype as between- and time (weeks) as within-subjects factors. Where appropriate, an independent T-test was performed. For the F19955, F13714 and S15535 dose-response experiments, a 2-way ANOVA for repeated measures was performed with dose as within-subject factor (5 levels) and genotype as between-subject factor (2 levels). Where appropriate one way-ANOVA with LSD post-hoc was performed. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows version 25 (LEAD technologies, Chicago, USA). Level of significance was set at $p < 0.05$.

3. Results

3.1 Sexual stability

The sexual performance of the selected experimental animal groups that exhibited a normal (1-2 ejaculations) and a low basal ejaculation frequency (0-1 ejaculation) during the 6 training days was registered and from the 63 male rats sexually trained from batch one and 32 from batch 2, only 24 and 21 animals (respectively) that showed normal and low sexual performance and ejaculations respectively, were selected to run the pharmacological studies. For the first batch, there was a significant week (time) effect $F_{(7,154)}$= 13.86, p<0.001, a significant week*genotype effect $F_{(7,154)}$=3.40, p<0.01 and a significant genotype effect ($F_{(1,22)}$= 23.81, p<0.001). In $SERT^{+/+}$ rats from week 3 onwards they performed significant more ejaculations (all p values are <0.05) compared to the first two weeks (fig.1 and table 1). In $SERT^{-/-}$ rats only week 16-20 significantly differed (all p values are <0.05) from all other weeks (fig 1, table 1). $SERT^{-/-}$ rats ejaculated significantly less compared to $SERT^{+/+}$ rats in week 3 (p<0.05), week 4 (p<0.05), week 5 (p<0.05), week 6 (p<0.05), week 7-14 (0.05), week 16-20 (p<0.01).

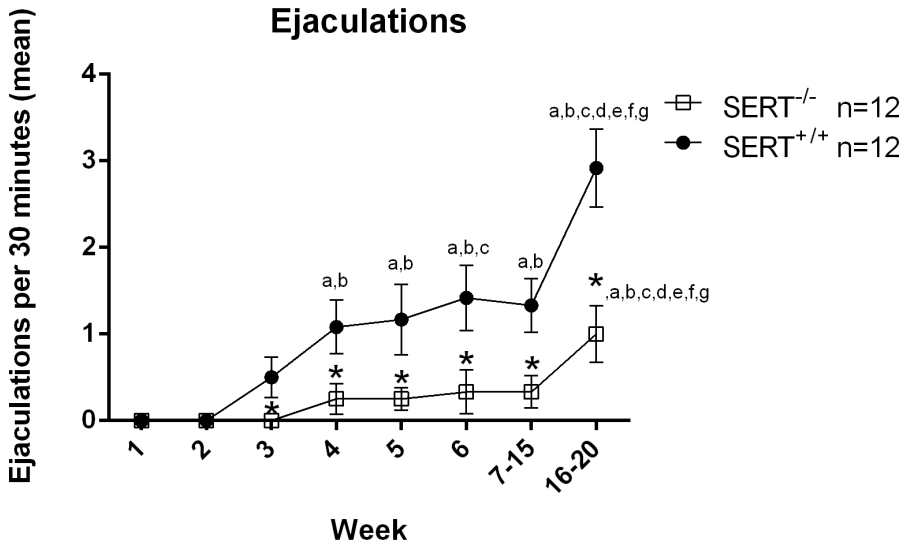

Fig 1. Mean ejaculation frequencies (±SEM) over 6 weeks of training of male Wistar rats of batch one. Added are also the mean ± SEM of the saline data from experiment one (F13714 and F15599) and two (S15535) of batch one. a: significantly different (*p<0.05*) from week 1;b: significantly different (*p<0.05*) from week 2;c: significantly different (*p<0.05*) from week 3; d: significantly different (*p<0.05*) from week 4; e: significantly different (*p<0.05*) from week 5; f: significantly different (*p<0.05*) from week 6; g: significantly different (*p<0.05*) from week 7-15; * significantly different (*p<0.05*) from $SERT^{+/+}$. Detailed statistical analyses are provided in table 1.

For the second batch of animals trained, there was a significant difference in weeks of training ($F_{(10,190)}$=3.32, p<0.001). In week 11-14 $SERT^{+/+}$ and $SERT^{-/-}$ rats had significant more ejaculations compared to all other weeks (all p values <0.01). No significant differences in time x genotype, and genotype effects were found during the training weeks (Figure 2, Table 2).

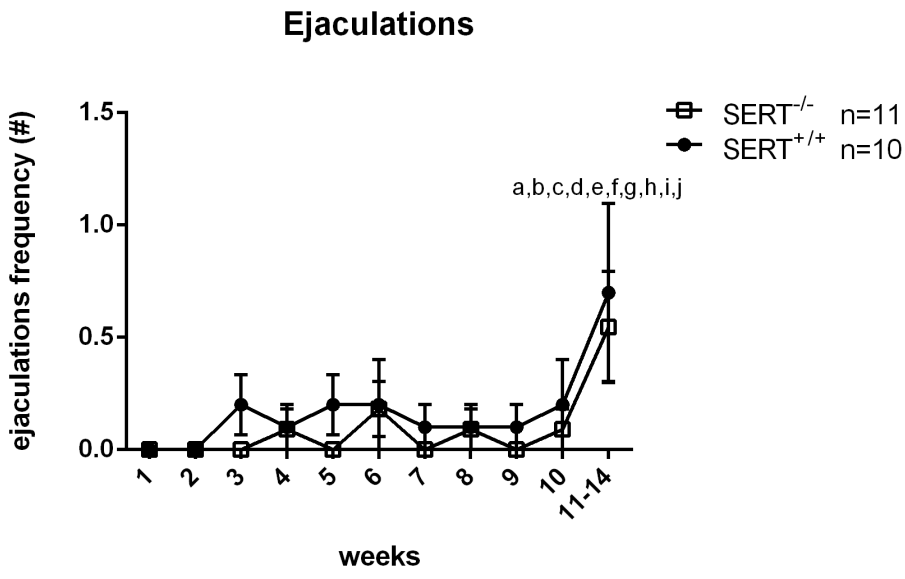

Fig 2. Mean ejaculation frequencies (±SEM) over 10 weeks of training of male Wistar rats of batch two. Added are also the mean ± SEM of the saline data from S15535 experiment (week 11-14). a: significantly different (*p<0.05*) from week 1;b: significantly different (*p<0.05*) from week 2;c: significantly different (*p<0.05*) from week 3; d: significantly different (*p<0.05*) from week 4; e: significantly different (*p<0.05*) from week 5; f: significantly different (*p<0.05*) from week 6; g: significantly different (*p<0.05*) from week 7; h: significantly different (*p<0.05*) from week 8; i: significantly different (*p<0.05*) from week 9; j: significantly different (*p<0.05*) from week 10; * significantly different (*p<0.05*) from $SERT^{+/+}$. Detailed statistical analyses are provided in table 2.

We included in fig. 1 the saline data gathered in the pharmacological experiments performed on batch one. The saline data obtained for all animals in weeks 7-15 were comparable to the last training data, but the saline data from the last (S15535) experiment (during week 16-20) showed significantly higher values. This 'enhanced' baseline level of sexual behavior made us decide (because of possible ceiling effects) to repeat the S15535 experiment in rats with very low levels of sexual ejaculation activity (batch two, data shown in table 2). In fig. 2 we also included the saline data gathered in the S15535 dose-response experiment of batch 2 (week 11-14). Again an enhanced baseline level of sexual behavior was found in the saline treated animals during the S-155355 treatment weeks.

3.2 Dose-response of F15599 (Fig.3 and table 3)

In the dose-response experiment a significant dose ($F_{(4,88)}$=8.75; p<0.001) and genotype ($F_{(1,22)}$=22.278; p<0.001) effect, but no interactions, were found for the number of ejaculations. Similar significances were found for ejaculation latencies and intromission ratios (see table 3 for statistics of all behavioural parameters). Further analysis revealed that the lowest and intermediate doses of F15599 (0, 0.01, 0.04 and 0.16 mg/kg) had no significant effects on sexual behavior in either genotype (fig 3, table 3). Compared to saline (p<0.001), 0.01 (p<0.01) and 0.04 (p<0.001) mg/kg F-15599, the highest dose (0.64 mg/kg) significantly increased the ejaculation frequency. Moreover, ejaculation latencies were significant shorter in 0.64 mg/kg F15599 compared to saline (p<0.01), 0.01 mg/kg (p<0.05) and 0.04 mg/kg (p<0.01) F15599 (fig. 3, table 3) in both $SERT^{+/+}$ and $SERT^{-/-}$ animals; the 0.64 mg/kg F15599 dose also significantly increased the efficiency of the animals to ejaculate (IR; p<0.05; fig 3, table 3) compared to saline (p<0.01), 0.01 mg/kg (p<0.05) and 0.04 mg/kg (p<0.05) F15599.

A significant decrease in the number of ejaculations of $SERT^{-/-}$ rats was found compared to $SERT^{+/+}$ rats in the saline treatment (p<0.05), and in the 0.01 mg/kg (p<0.05), 0.04 mg/kg (p<0.05), 0,16 mg/kg (p<0.05), and 0.64 mg/kg (p<0.05) F15599 treatment. Similarly, an increase in ejaculation latency was found for $SERT^{-/-}$ rats compared to $SERT^{+/+}$ rats in saline treatment (p<0.001), and in 0.01 mg/kg (p<0.01), 0.04 mg/kg (p<0.001), 0,16 mg/kg (p<0.05), and 0.64 mg/kg (p<0.001) F15599 treatment. For the intromission ratio, a significant decrease was found for $SERT^{-/-}$ rats compared to $SERT^{+/+}$ rats in saline treatment (p<0.05), and in the 0.01 mg/kg (p<0.01), 0.04 mg/kg (p<0.05), and 0,16 mg/kg (p<0.05) F15599 treatment.

3.3 Dose-response of F13714 (Fig. 4; table 4)

Overall, F13714 induced pro-sexual effects in both genotypes, although the dose-effect curves for both genotypes differed considerably (fig.4, table 4). Considering the ejaculations, a significant dose ($F_{(4,88)}$= 3.287, p<0.05), genotype ($F_{(1,22)}$= 20.649, p<0.001), and genotype x dose interaction ($F_{(4,88)}$= 4.810, p<0.01) was found. Similar significances were found for ejaculation latencies (see table 4 for statistics of all behavioural parameters). In $SERT^{+/+}$ rats, F13714 stimulated sexual behavior significantly, as illustrated (compared to saline) in the increase in the ejaculation frequency at 0.0025 mg/kg (p<0.01), 0.01 mg/kg (p=0.06) and 0.04 mg/kg (p<0.05) mg/kg F13714). In the $SERT^{-/-}$ rats, pro-sexual effects were observed only at the highest dose (0.16mg/kg) versus saline (p<0.05) and 0.025 mg/kg (p<0.05) F13714. Although the ejaculation latency was decreased at this high dose for both genotypes, this difference was not statistically significant. The number of mounts were equally decreased in $SERT^{+/+}$ and $SERT^{-/-}$ rats at 0.16 mg/kg F13714 compared to saline (p<0.01), 0.025 mg/kg (p<0.01), 0.01 mg/kg (p<0.001) and 0.04 mg/kg (p<0.05) F13714. In

$SERT^{+/+}$, but not in $SERT^{-/-}$, rats, the intromission latency increased at the highest dose ($F(_{4.55})$= 4.203, p<0.01). The intromission latency at the highest dose (0.16 mg/kg) of F13714 was significant longer compared to saline (p<0.01), 0.0025 mg/kg (p<0.001), 0.01 mg/kg (p<0.01), and 0.04 mg/kg (p<0.05) F13714. Lastly, the number of intromissions were significantly decreased in $SERT^{+/+}$ rats only ($F(_{4.55})$= 8.194; p<0.001). Intromissions were significant reduced in animals treated with 0.16 mg/kg F13714 compared to those treated with saline (p<0.001), , 0.0025 mg/kg (p<0.01) and 0.01 mg/kg (p<0.01) F13714 treated $SERT^{+/+}$ rats. In addition, 0.04 mg/kg F13714 treated $SERT^{+/+}$ rats had a significant reduced number of intromissions compared to those treated with saline (p<0.001), 0.0025 mg/kg (p<0.01), and 0.01 mg/kg (p<0.05) F13714.

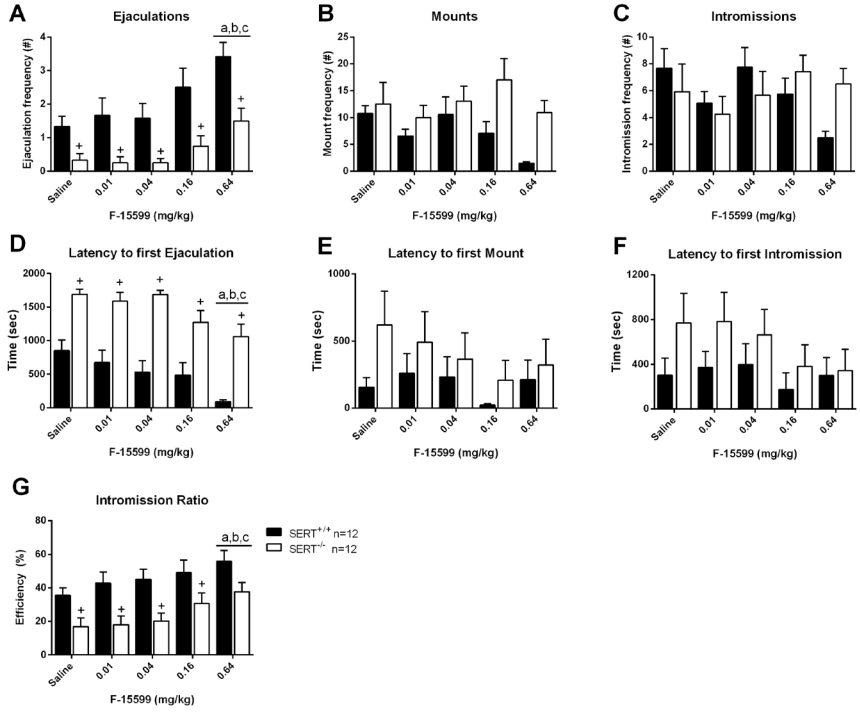

Fig 3. Sexual behavior of male rats treated with 0, 0.01, 0.04, 0.16 or 0.64 mg/kg, IP of F-15599. The number and latency of ejaculations per 30 min (A, D), number and latency of Mounts (B, E), number and latency of Intromissions (C, F) and Intromission Ratio (G) of the first Ejaculation Series are displayed. Detailed statistical analyses are provided in Table 3. a: significant difference (p<0.05) compared to saline group, b: significant difference (p<0.05) compared to 0.01mg/kg group, c: significant difference (p<0.05) compared to 0.04/mg/kg group. +: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ (p<0.05).

$SERT^{-/-}$ rats had significant lower ejaculation frequencies compared to $SERT^{+/+}$ rats after treatment with saline ($p<0.05$), and after treatment with 0.0025 mg/kg ($p<0.01$), 0.01 mg/kg ($p<0.01$), and 0.04 mg/kg ($p<0.01$) F13714. For mounts, only at a dose of 0.04 mg/kg F13714 $SERT^{-/-}$ rats showed a significant higher mount frequency ($p<0.01$) compared with $SERT^{+/+}$ rats. At the same dose $SERT^{-/-}$ rats also showed a higher intromission frequency compared to $SERT^{+/+}$ rats. For latency to the first ejaculation a significant increase was found for $SERT^{-/-}$ rats compared to $SERT^{+/+}$ rats for all doses (p values are all <0.01). The latency to the first mount was significantly higher for $SERT^{-/-}$ rats compared to $SERT^{+/+}$ after saline treatment ($p<0.05$) and the latency to the first intromission was also significantly higher for $SERT^{-/-}$ rats compared to $SERT^{+/+}$ at 0.0025 mg/kg F13714 ($p<0.05$).

3.4 Dose-response of S15535 (Fig. 5 and 6; tables 5 and 6)

S15535 (0.25, 1, and 4-mg/kg) had no significant effects on sexual behavior in $SERT^{+/+}$ and $SERT^{-/-}$ (fig. 5 and 6) compared to saline in either batch of animals. In the first batch of rats, a significant genotype effect for ejaculation frequencies was found ($F(_{1,22})=21.167$, $p<0.001$; fig. 5A). $SERT^{+/+}$ rats had significant higher ejaculation frequencies after treatment with saline ($p<0.001$), 0.25mg/kg ($p<0.001$), 1mg/kg ($p<0.05$) and 4mg/kg ($p<0.001$) S15535in comparison with $SERT^{-/-}$ rats () . Similar effects were found for the ejaculation latency ($F(_{1,22})=25.627$, $p<0.001$; fig. 5D; table 5) where there was an increase for $SERT^{-/-}$ versus $SERT^{+/+}$ animals after saline treatment ($p<0.001$), and after treatment with 0.25mg/kg ($p<0.05$), 1 mg/kg ($p<0.05$) and 4 mg/kg ($p<0.001$) S15535, and to some extent in the intromission ration, although this was only significant in the saline treated ($p<0.05$) and 4 mg/kg ($p<0.05$) S155355 treated group. In the second batch of animals, no significant differences were found in the majority of the parameters measured, although a significant dose effect was found for the number of mounts ($F(_{3,80})=2.946$, $p<0.05$). Analysis revealed a significant reduction in the number of mounts between saline and 4 mg/kg S-155355 ($p<0.05$) and between 1 mg/kg and 4 mg/kg S-155355 ($p<0.05$). In addition, a genotype effect was found in the latency to the first intromission ($F(_{1,19})=6.499$, $p<0.05$). Compared to $SERT^{+/+}$, $SERT^{-/-}$ displayed a shorter latency to the first intromission ($p<0.05$; see fig.6 and Table 6).

3.5 Comparison between F15599 and F13714 (supplementary figure 1)

A fit curve plot for $SERT^{+/+}$ and $SERT^{-/-}$ rats on a log scale (see supplementary figure 1) was made where data were normalized against the saline treated group. The ED50 was calculated for $SERT^{+/+}$ (F15599, ED50 = 0.21; F13714, ED50= 0.0065) and $SERT^{-/-}$ (F15599, ED50 = 0.165; F13714, ED50= 0.00178) and showed that F13714 was more potent compared to F15599 in both $SERT^{+/+}$ and $SERT^{-/-}$ rats. The curved fit plot also showed that $SERT^{-/-}$ rats were sensitive to both compounds, as they were able to increase the percentage of ejaculations compared with the saline treated group.

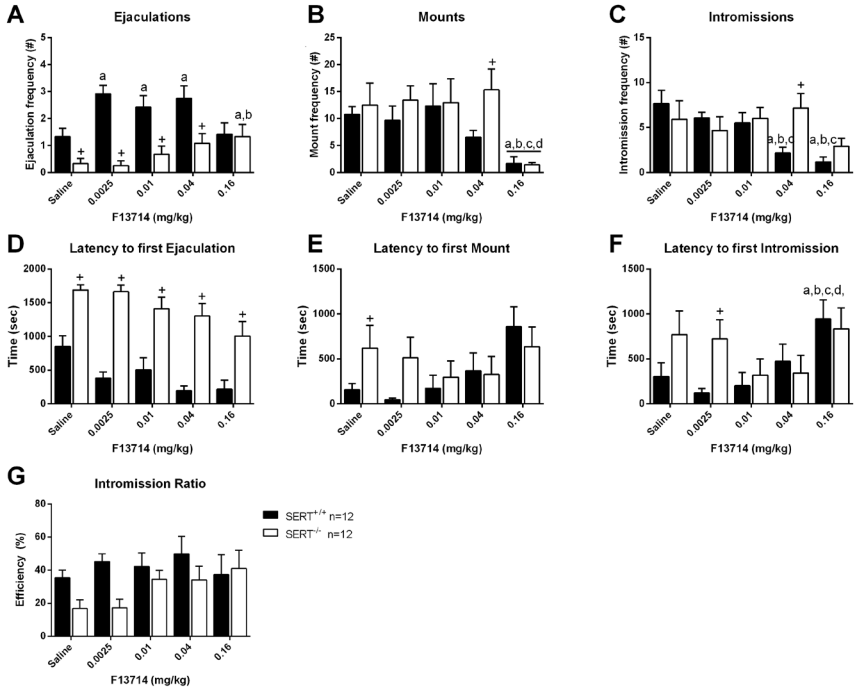

Fig 4. Sexual behavior of male rats treated with 0, 0.0025, 0.01, 0.04 or 0.16 mg/kg F13714. The number and latency of ejaculations per 30 min (A, D), number and latency of Mounts (B, E), number and latency of Intromissions (C, F) and Intromission Ratio (G) of the first Ejaculation Series are provided. Detailed statistical analyses are displayed in Table 4. a: significant difference ($p<0.05$) compared to saline group, b: significant difference ($p<0.05$) compared to 0.0025mg/kg group, c: significant difference ($p<0.05$) compared to 0.01/mg/kg group. +: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ ($P<0.05$).

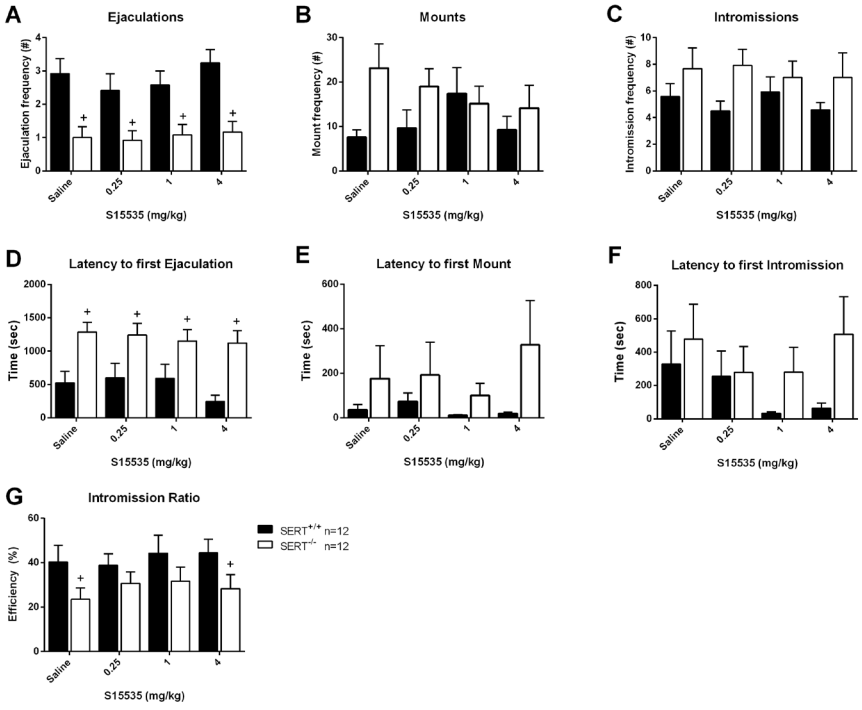

Fig 5. Sexual behavior of male rats from batch one treated with 0, 0.25, 1 or 4 mg/kg S15535. The number and latency of ejaculations per 30 min (A, D), number and latency of Mounts (B, E), number and latency of Intromissions (C, F), post-ejaculatory interval (G) and Intromission Ratio (H) of the first Ejaculation Series are provided. Detailed statistical analyses are displayed in Table 5. +: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ groups.

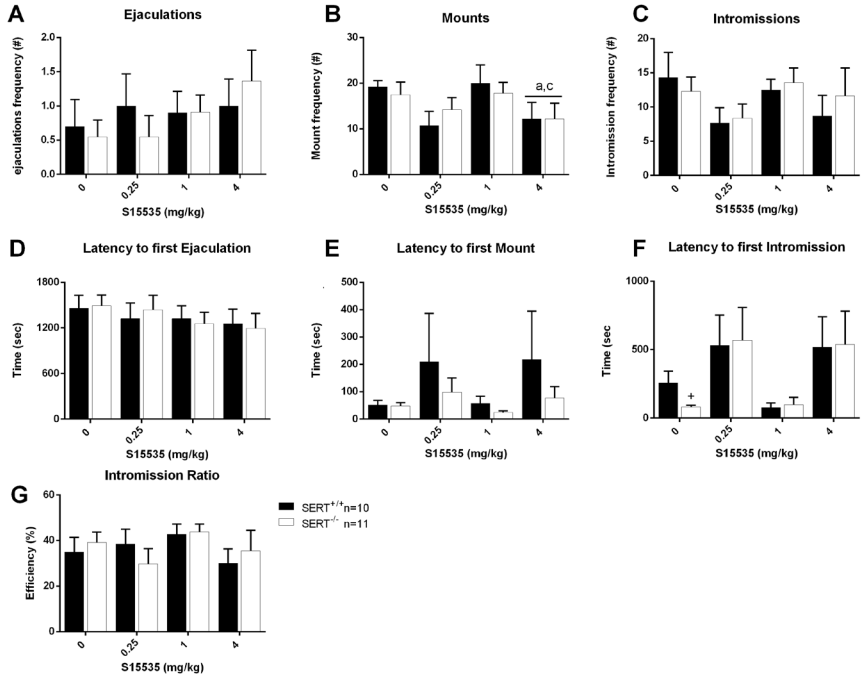

Figure 6. Sexual behavior of male rats from batch two treated with 0, 0.25, 1 or 4 mg/kg S15535. The number and latency of ejaculations per 30 min (A, D), number and latency of Mounts (B, E), number and latency of Intromissions (C, F) and Intromission Ratio (G) of the first Ejaculation Series are given. Detailed statistical analyses are shown in Table 6. a: significant difference ($p<0.05$) compared to saline group, c: significant difference ($p<0.05$) compared to 1/mg/kg group. +: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ ($P<0.05$).

4. Discussion

In the present study, after extensive training of the two genotypes studied ($SERT^{+/+}$ and $SERT^{-/-}$), animals showed two different but stable sexual phenotypes, confirming earlier findings (Chan et al., 2011) where male $SERT^{+/+}$ rats performed sexual behavior at a higher level than $SERT^{-/-}$ rats. Permanent changes in serotonergic processes in the central nervous system by removing the SERT protein from conception on (Chan et al., 2011; Olivier et al., 2011), apparently leads to permanent changes in overt male sexual behavior in rats. The male rat sexual behavior paradigm used in the present studies has been developed over the last decades (Chan et al., 2008; Berend Olivier et al., 2010; Pattij et al., 2005), specifically to test the effects of psychoactive drugs, including antidepressants (Chan et al., 2010; Heijkoop et al., 2018; Waldinger & Olivier, 2005). The paradigm is able to distinguish acute effects of drugs like the pro-sexual effects of 5-HT_{1A} R agonist (Pattij et al., 2005), but also the chronic inhibitory effects of SSRI antidepressants (Bijlsma et al., 2014; Chan et al., 2011; Chan et al., 2010). Pro-sexual effects of drugs in male rat sexual behavior are reflected in the speed of onset of sexual activity towards a newly introduced female in behavioral estrus; reflected in a shorter interval to reach ejaculation (Andersson & Larsson, 1994), including reduced number of mounts and intromissions to reach ejaculation and enhanced number of ejaculations over a certain test period (in our case 30 min). Reduction of sexual behavior, e.g. by chronic antidepressants (Bijlsma et al., 2014; Chan et al., 2010) has reversed effects. This chronic SSRI (antidepressant)-induced profile of reduced male sexual behavior is comparable to the sexual behavior of $SERT^{-/-}$ rats and supports the hypothesis that male $SERT^{-/-}$ rats are modeling the sexual effects of chronic SSRI administration (Chan et al., 2011; Berend Olivier et al., 2010).

Two biased 5-HT_{1A} R agonists, the preferential 5-HT_{1A} auto-receptor agonist F13714 (Assié et al., 2006; Becker et al., 2016) and the preferential 5-HT_{1A} heteroreceptor agonist F15599 (Becker et al., 2016; A Newman-Tancredi et al., 2009) were tested in $SERT^{+/+}$ and $SERT^{-/-}$ rats. Both compounds induced pro-sexual activity in $SERT^{+/+}$ and $SERT^{-/-}$ rats. F13714 is considerably more potent than F15599 in eliciting the pro-sexual effects, but the similarity of the response of both compounds on male sexual behavior suggests that both compounds share comparable mechanisms of action in evoking this behavior. This may point to an autoreceptor-mediated effect. Unfortunately, full dose-response curves of this prosexual effect were not available for both compounds making definite conclusions impossible. In F13714-treated $SERT^{-/-}$ rats the dose-response curve was shifted to the right compared to the $SERT^{+/+}$ rats, but this was not the case in F15599 rats where the sexual inhibiting doses were comparable in both genotypes. 5-HT_{1A} receptor stimulation by 'non-selective' (with regard to pre- and postsynaptic receptors) 5-HT_{1A} R agonists like 8-OH-DPAT, flesinoxan, buspirone, ipsapirone and others (Olivier et al., 1999) have pro-sexual effects in wildtype

rats (Snoeren et al., 2014 for review), but no studies were performed before, in which the specific contributions of 5-HT_{1A} auto-receptors or 5-HT_{1A} heteroreceptors (or both) are investigated. S15535, an auto-receptor selective 5-HT_{1A} R agonist and heteroreceptor-selective 5-HT_{1A} R antagonist, did not have any effects on male sexual behavior of $SERT^{+/+}$ and $SERT^{-/-}$ rats, neither in normal ejaculating (on average 1-2 ejaculations/30 min, batch 1) nor sluggish (0-1 ejaculations/30 min, batch 2) rats. We conclude that S15535 behaves as a 'silent' 5-HT_{1A} receptor ligand in male rat sexual behavior.

The prototypal 5-HT_{1A} R agonist (+/-) or (+)-8-OH-DPAT, a nonselective auto-receptor and heteroreceptor agonist (Larsson et al., 1990), has strong and dose-dependent pro-sexual effects (Chan et al., 2011; Mos et al., 1991). This pro-sexual effect can be fully antagonized by the 5-HT_{1A} R antagonist WAY100,635, a behaviorally silent compound (T. R. de Jong & Neumann, 2015). In male $SERT^{-/-}$ rats (Chan et al., 2011) 8-OH-DPAT had pro-sexual effects, although (like the biased agonist F13714 in the present study) the dose-response curve was shifted to the right compared to $SERT^{+/+}$ rats. The lack of any behavioral effect of S15535 in either $SERT^{+/+}$ or $SERT^{-/-}$ rats is rather puzzling. Apparently, 5-HT_{1A} R antagonistic activity on 5-HT_{1A} heteroreceptors in $SERT^{-/-}$ rats did not cause inhibition of male sexual behavior like WAY100,635 treatment (Chan et al., 2011). The stimulating effect of F13714 and F15599 in male sexual behavior in both $SERT^{+/+}$ and $SERT^{-/-}$ rats is also quite puzzling, because it makes explanations in term of pre- or postsynaptic 5-HT_{1A} receptor mechanisms involved, troublesome. However, it remains possible that the preferential postsynaptic 5-HT_{1A} R agonist F15599 at higher doses (like in this experiment) also displays some presynaptic autoreceptor agonistic activity. In that case F15599 does not appear the specific tool to selectively activate postsynaptic 5-HT_{1A} heteroreceptors.

How do the sexual data obtained with these three serotonergic ligands compare to their effects in other behavioral systems? The research group of De Boer (De Boer & Newman-Tancredi, 2016) has tested these (and other) ligands extensively in male rat models of offensive aggression in Wildtype Groningen (WTG) rats. In male rat offensive aggression (De Boer et al.,1999; 2000) 8-OH-DPAT potently and dose-dependently reduced offensive aggression but also induces strong sedative-like behaviors. Because 5-HT_{1A} R agonists induce a so-called serotonin-5-HT_{1A} syndrome, characterized by Lower Lip Retraction (LLR), Forepaw Treading (FPT) and Flat Body Posture (FBP), it is not completely clear whether this sedative-like activity is similar to these serotonergic behaviors. These anti-aggressive and other effects of 8-OH-DPAT can be fully antagonized by WAY100,635 (De Boer et al., 1999; De Boer et al., 2000), a silent antagonist in offensive aggression. F13714, F15599 and S15535 all reduce offensive aggression (De Boer & Newman-Tancredi, 2016). Both F13714 and F15599 induce a serotonergic-5-HT_{1A} syndrome in rats (Assié et al., 2010; Jastrzębska-Więsek et al., 2018; Newman-Tancredi et al., 2009). S15535 does not induce

the serotonergic-5-HT_{1A} syndrome at all (De Boer & Newman-Tancredi, 2016; Jastrzębska-Więsek et al., 2018) and also has no sedative-like activity in offensive aggression (De Boer et al., 2000). WAY100,635 antagonized the anti-aggressive action of S15535, F15599 and F13714 (De Boer & Newman-Tancredi, 2016).

If the mechanisms of action of the three 5-HT_{1A} ligands as extensively investigated by various research groups are true, mechanistic explanations of the behavioral effects found in male sexual behavior are rather difficult to explain. Serotonergic 5-HT_{1A} auto-receptors in the raphe nuclei are generally considered as, upon activation, leading to inhibition of cell firing and consequently a decrease of serotonin release. Subsequently, all postsynaptic 5-HT (hetero) receptors (including 5-HT_{1A} heteroreceptors) receive diminished or no stimulation by serotonin and depending on the coupling of the postsynaptic receptor to different transduction mechanisms the neuron involved will be activated or inhibited. In case of a non-selective 5-HT_{1A} receptor agonist like 8-OH-DPAT, next to its inhibiting action on the serotonergic neuron, direct 5-HT_{1A} heteroreceptor stimulation still occurs leading to post-synaptically mediated effects, like the serotonergic-5-HT_{1A} behavioral syndrome (Berendsen et al., 1990; Jastrzębska-Więsek et al., 2018). In the case of F13714, a relatively selective (compared to heteroreceptor) 5-HT_{1A} auto-receptor agonist (Assié et al., 2006) potently facilitated sexual activity in male $SERT^{+/+}$ rats suggesting that pro-sexual activity is related to activation of 5-HT_{1A} auto-receptors. The relatively selective 5-HT_{1A} heteroreceptor agonist F15599 also facilitated male sexual activity in $SERT^{+/+}$ rats. The difference in potency (factor 256 difference) to obtain the pro-sexual activity (at the lowest effective dose) can possibly be explained by the difference of the in vitro and in vivo affinity and efficacy of both compounds on 5-HT_{1A} receptors (Assié et al., 2010; Jastrzębska-Więsek et al., 2018; Newman-Tancredi, 2011b). This might be taken as suggestive that both compounds exert pro-sexual activity via activation of 5-HT_{1A} auto-receptors. Strangely enough, both compounds also activate serotonergic-5-HT_{1A} syndrome (Becker et al., 2016; Newman-Tancredi, 2011b). The 5-HT_{1A} auto-receptor agonist S15535 does not induce pro-sexual behavior, neither in normal nor in sexually sluggish behaving rats. Whether blocking of postsynaptic 5-HT_{1A} heteroreceptors antagonizes the expected pro-sexual effect of the auto-receptor stimulation is rather difficult to envisage. This would assume a rather high level of basal activity of 5-HT_{1A} heteroreceptors involved in sexual behavior. Interestingly, Pattij et al. (2005) showed that sluggish, normal and rapid ejaculators showed increased ejaculations after treatment with 8-OH-DPAT, however when rats were re-tested 1 week after this 5-HT_{1A} R agonist administration all phenotypes returned to ejaculatory behavior levels found before the 8-OH-DPAT treatment. In the present study we found that during the weeks where treatment with S155355 were administered, the saline groups (and thus baseline levels) showed significant higher ejaculation frequencies compared to the ejaculation frequencies during the training weeks. This might suggest

that pro-sexual effects due to 5-HT_{1A} R agonist can be long-lasting, most likely due to alterations in the 5-HT_{1A} receptors. Further research is warranted to investigate how long this effect would persist and whether 1 week after all treatments with 5-HT_{1A} R agonists, and without saline treatment, is still present.

$SERT^{-/-}$ rats, a model of permanently changed serotonergic activity in the brain (Homberg et al., 2007) and associated with an altered sexual phenotype (Chan et al., 2011) may be helpful in explaining the behavioral effects found for the three compounds. Chan et al. (2011) have found that 8-OH-DPAT has pro-sexual effects in male $SERT^{-/-}$ rats, although the dose-response curve has been shifted to the right compared to $SERT^{+/+}$ rats. Remarkably, WAY100,635, a non-selective 5-HT_{1A} receptor antagonist and without any behavioral effects in $SERT^{+/+}$ males, was (dose-dependently) inhibitory in $SERT^{-/-}$ rats. WAY100,635 was able to completely antagonize the pro-sexual effects of 8-OHDPAT in $SERT^{+/+}$ rats but only partially in $SERT^{-/-}$ rats (Chan et al., 2011). We concluded from these data that complete absence of SERT molecules had led to alterations in 5-HT_{1A} receptor functioning, hypothesizing that one pool of 5-HT_{1A} receptors mediates pro-sexual effects of 5-HT_{1A} receptor stimulation and is not (de)sensitized, whereas another pool of 5-HT_{1A} receptors, mediating the inhibitory effects of antagonized 5-HT_{1A} receptors seems sensitized in the $SERT^{-/-}$ rats. The hypothesis of two differentially regulated 5-HT_{1A} receptor pools in $SERT^{-/-}$ rats has also been found in autonomic regulation of body temperature and stress (Olivier et al., 2008). The findings with F15599 and F13714 in the $SERT^{-/-}$ rats cannot be explained in terms of action on different 5-HT_{1A} receptor pools. If any, both compounds seem to activate the pool mediating the pro-sexual effects. The 5-HT_{1A} heteroreceptor antagonistic effects of S15535 do not lead to inhibition of male sexual behavior in the better performing (normal ejaculating) $SERT^{-/-}$ rats, as was the case for WAY100,635 in the Chan et al. (2011) study.

Our expectation that biased 5-HT_{1A} receptor agonists and a mixed 5-HT_{1A} presynaptic receptor agonist and postsynaptic antagonist might help to reveal the potential contribution of these different 5-HT_{1A} receptors was too optimistic. The mechanisms of action of the respective molecules are probably, especially in vivo in complicated networks, where 5-HT_{1A} receptors interact with various other neurotransmitter systems in the modulation of male sexual behavior, to complex and need more research.

5.Conclusion

The data collected with the pharmacological experiments show that selective (preferential) pre- and post-synaptic 5-HT_{1A} R agonists possess pro-sexual effects in $SERT^{+/+}$ and $SERT^{-/-}$, although the response is diminished in $SERT^{-/-}$ animals, most likely due to desensitization of 5-HT_{1A} receptors. The pharmacological experiment with S15535 compared with previous experiments performed in aggression, shows that even though aggression and sexual behavior share most of their neurobiological substrate, at least at the 5-HT_{1A} R level we are dealing with separate neurobiological substrates for male sexual and aggressive behavior in rats, but further experiments are needed to support this idea.

6. Acknowledgments

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7.Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8.Authors Contribution Statement

DCEF, BO and JDAO contributed with conception and design of the work, DCEF carried out all the experimental work, data collection and analysis and work draft. DCEF, BO, SFdD and JDAO contributed to the interpretation of the data and results, made sure all parts of the work were appropriately investigated and resolved. DCEF, BO, SFdB, MDW and JDAO contributed on revising critically the intellectual content, accountability and accuracy of the work. DCEF, BO, SFdB MDW and JDAO provided approval for the publication of the content.

Tables

Table 1: Sexual Behavior performance during training weeks of male SERT$^{+/+}$ and SERT$^{-/-}$ Wistar rats from batch 1. N=12/group

	Week			
	1	2	3	4
SERT	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
	A	**B**	**C**	**D**
+/+	0.0±0.0	0.0±0.0	0.50±0.23	1.08±0.31 **A,B**
-/-	0.0±0.0	0.0±0.0	0.0±0.0	0.25±0.17
T-test genotype per week	**ns**	**ns**	* $T_{(1,22)}$=2.171, p<0.05	* $T_{(1,22)}$=2.311, p<0.05
2-WAY ANOVA repeated measures	Time (week) effect $F_{(7,154)}$=13.855, p<0.001 Time(week)*Genotype effect $F_{(7,154)}$=3.396, p<0.01 Genotype effect $F_{(1,22)}$=23.807, p<0.001			

A: significantly different from week 1; B: significantly different from week 2; C: significantly different from week 3; D: significantly different from week 4;

Table 2: Sexual Behavior performance during training weeks of male SERT$^{+/+}$ and SERT$^{-/-}$ Wistar rats from batch 2. N=10 and N=11 respectively.

	Week				
	1	2	3	4	5
SERT	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
	A	**B**	**C**	**D**	**E**
+/+	0.0±0.0	0.0±0.0	0.20±0.13	0.10±0.10	0.20±0.13
-/-	0.0±0.0	0.0±0.0	0.0±0.0	0.09±0.09	0.0±0.0
T-test genotype per week	**NA**	**NA**	**NA**	**NA**	**NA**
2-WAY ANOVA repeated measure	Time (week) effect $F_{(10,190)}$=3.321, p<0.001 No Time(week)*Genotype effect $F_{(10,190)}$=0.607, n.s. No Genotype effect $F_{(1,19)}$=2.072, n.s.				

A: significantly different from week 1; B: significantly different from week 2; C: significantly different from week 3; D: significantly different from week 4; E: significantly different from week 5;

	Week				
	5 Mean ± SEM **E**	6 Mean ± SEM **F**	7-15 Mean ± SEM **G**	16-20 Mean ± SEM H	One Way ANOVA Time effect
	1.16±0.40 **A,B**	1.41± 0.37 **A,B,C**	1.33±0.30 **A,B**	2.91±0.45 **A,B,C,D,E,F,G**	$F_{(7,88)}$=9.37; p<0.001
	0.25±0.13	0.33±0.25	0.33±0.18	1.00±0.32 **A,B,C,D,E,F,G**	$F_{(7,88)}$=3.25; p<0.01
	*	*	*	*	
	$T_{(1,22)}$=2.154, p<0.05	$T_{(1,22)}$=2.370, p<0.05	$T_{(1,22)}$=2.760, p<0.05	$T_{(1,22)}$=3.443, p<0.01	

E: significantly different from week 5; F: significantly different from week 6; G: significantly different from week 7-15; all *p* values are *<0.05*.

	Week						
	6 Mean ± SEM **F**	7 Mean ± SEM **G**	8 Mean ± SEM **H**	9 Mean ± SEM **I**	10 Mean ± SEM **J**	11-14 Mean ± SEM K	One Way ANOVA Time effect
	0.20±0.20	0.10±0.10	0.10±0.10	0.10±0.10	0.20±0.20	0.70±0.39	
	0.18±0.12	0.0±0.0	0.09±0.09	0.0±0.0	0.09±0.09	0.54±0.24 **A,B,C,D,E,F,G,H,I,J**	
	NA	**NA**	**NA**	**NA**	**NA**	**NA**	$F_{(10,220)}$=3.418, p<0.001

F: significantly different from week 6; G: significantly different from week 7; H: significantly different from week 8; I: significantly different from week 9; J: significantly different week 10; all *p* values are *<0.01*.

Table 3: Effects of F15599 on Sexual Behavior of male SERT$^{+/+}$ and SERT$^{-/-}$ Wistar rats. N=12/group

Dose of F-15599, mg/kg	0 mg/kg A	0.01 mg/kg B
Parameters measured	Mean ±SEM	Mean ±SEM
# E SERT +/+	1.3±0.30	1.6±0.51
SERT -/-	0.3±0.18	0.25±0.17
One-way ANOVA between genotype per dose	* $F(_{1,22})$= 7.615, p<0.05	* $F(_{1,22})$= 6.807, p<0.05
2-WAY ANOVA repeated measures	Dose effect $F(_{4,88})$=8.747, p<0.001 No Dose*Genotype effect $F(_{4,88})$=0.594, n.s. Genotype effect $F(_{1,22})$=22.278, p<0.001	
# M 1st series		
SERT +/+	10.75±1.457	6.53±1.22 1
SERT -/-	12.50±4.061	0.00±2.25
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	Dose effect $F(_{4,88})$=5.334, p<0.001 No Dose*Genotype effect $F(_{4,88})$=2.030, n.s. No Genotype effect $F(_{1,22})$=2.962, n.s.	
# I 1st series		
SERT +/+	7.66±1.46	5.08±0.84
SERT -/-	5.91±2.07	4.25±1.32
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F(_{4,88})$=1.707, n.s No Dose*Genotype effect $F(_{4,88})$=2.114, n.s. No Genotype effect $F(_{1,22})$=0.856, n.s	
Latency 1st E (s) SERT +/+	853.3±157.3	673.8±184.6
SERT -/-	1689.4±75.8	1589±131.2
One-way ANOVA between genotype per dose	* $F(_{1,22})$= 22.922, p<0.001	* $F(_{1,22})$= 16.335, p<0.01
2-WAY ANOVA repeated measures	Dose effect $F(_{4,88})$=7.446, p<0.001 No Dose*Genotype effect $F(_{4,88})$=0.525, n.s. Genotype effect $F(_{1,22})$=77.110, p<0.001	

	0.04 mg/kg C	0.16 mg/kg D	0.64 mg/kg E	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	Mean ±SEM	
	1.58±0.43 0.25±0.136 * $F_{(1,22)}$= 8.638, p<0.05	2.50±0.57.7.5 0±0.30 * $F_{(1,22)}$= 7.317, p<0.05	3.41±0.41 1.50±0.37 A,B,C * $F_{(1,22)}$= 11.569; p<0.05	 $F_{(4,115)}$=5.286, p<0.001
	10.58±3.23 13.08±2.77 NA	7.08±2.15 17.00±3.97 NA	1.50±0.26 10.92±2.26 NA	NA
	7.75±1.45 5.66±1.76 NA	5.75±1.16 7.41±1.22 NA	2.50±0.46 6.50±1.14 NA	 NA
	528.6±173.0 1686±62.3 * $F_{(1,22)}$= 39.601, p<0.001	487.3±183.6 1272±177.2 * $F_{(1,22)}$= 9.466, p<0.05	88.67±29.8 1063±184.9 A,B,C * $F_{(1,22)}$= 27.033, p<0.001	 $F_{(4,115)}$= 3.010; p<0.05

Table 3: Continued

Dose of F-15599, mg/kg	0 mg/kg **A**	0.01 mg/kg **B**
Parameters measured	Mean ±SEM	Mean ±SEM
Latency 1st M (s) SERT +/+	155.5±71.6	260.0±146.7
SERT -/-	619.6±251.7	491.3±228.4
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(4,88)}$=1.260, n.s. No Dose*Genotype effect $F_{(4,88)}$=1.260, n.s. No Genotype effect $F_{(1,22)}$=1.906, n.s.	
Latency 1st I (s)		
SERT +/+	302.6±152.4	371.3±145.3
SERT -/-	770.1±262.5	782.6±259.4
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(4,88)}$=1.418, n.s. No Dose*Genotype effect $F_{(4,88)}$=0.532, n.s. No Genotype effect $F_{(1,22)}$=2.252, n.s.	
IR_1 SERT +/+	35.5±4.4	42.8±6.6
SERT -/-	16.8±5.1	18.0±5.2
One-way ANOVA between genotype per dose	* $F_{(1,22)}$= 7.501, p<0.05	* $F_{(1,22)}$= 8.580, p<0.01
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=4.566, p<0.01 No Dose*Genotype effect $F_{(4,88)}$=0.205, n.s. Genotype effect $F_{(1,22)}$=19.573, p<0.001	

A: significant difference compared to saline group, B: significant difference compared to 0.01mg/kg group, C: significant difference compared to 0.04/mg/kg group,

	0.04 mg/kg C	0.16 mg/kg D	0.64 mg/kg E	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	Mean ±SEM	
	232.2±150.7 365.1±195.6 NA	23.8±8.8 208.0±147.8 NA	211.9±146.8 322.3±192.9 NA	 NA
	397.1±186.1 663.7±226.4 NA	382.1±148.2 252.4±193.2 NA	300.2±159.2 342.8±191.0 NA	 NA
	45.0±6.2 20.2±4.7 * $F_{(1,22)}$= 9.943, p<0.05	49.0±7.4 30.7±6.1 * $F_{(1,22)}$= 3.608, p<0.05	55.9±6.4 37.6±5.6 A,B,C ns	 $F_{(4,115)}$= 3.111,p<0.05

p values set at <0.05 (for specific p values go to results sections). *: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ (p<0.05).

Table 4: Effects of F13714 on Sexual Behavior of male SERT$^{+/+}$ and SERT$^{-/-}$ Wistar rats. N=12/group

Dose of F-13714, mg/kg	0 mg/kg **A**	0.0025 mg/kg **B**
Parameters measured	Mean ±SEM	Mean ±SEM
# E SERT +/+	1.33±0.30	2.91±0.31 **A**
SERT -/-	0.33±0.18	0.25±0.17
One-way ANOVA between genotype per dose	*	*
	$F_{(1,22)}$=7.615, p<0.05	$F_{(1,22)}$=54.680, p<0.01
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=3.287, p<0.05 Dose*Genotype effect $F_{(4,88)}$=4.810, p<0.01 Genotype effect $F_{(1,22)}$=20.649, p<0.001	
# M 1st series		
SERT +/+	10.7±1.45	9.6±2.68
SERT -/-	12.5±4.06	13.4±2.63
One-way ANOVA between genotype per dose	**ns**	**ns**
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=2.285, p=0.06 No Dose*Genotype effect $F_{(4,88)}$=1.294, n.s. Genotype effect $F_{(1,22)}$=6.943, p<0.05	
# I 1st series		
SERT +/+	7.6±1.46	6.0±0.60
SERT -/-	5.9±2.07	4.6±1.53
One-way ANOVA between genotype per dose	**ns**	**ns**
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=5.308, p<0.01 Dose*Genotype effect $F_{(4,88)}$=3.128, p<0.05 No Genotype effect $F_{(1,22)}$=0.510, n.s.	
Latency 1st E (s) SERT +/+	853.3±157.3	379.3±91.56
SERT -/-	1689.4±75.8	1664±97.59
One-way ANOVA between genotype per dose	*	*
	$F_{(1,22)}$=22.922, p<0.001	$F_{(1,22)}$=92.148, p<0.001
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=7.604, p<0.001 Dose*Genotype effect $F_{(4,88)}$=1.253, p<0.05 Genotype effect $F_{(1,22)}$=62.797, p<0.001	

	0.01 mg/kg C	0.04 mg/kg D	0.16 mg/kg E	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	Mean ±SEM	
	2.41±0.43 A	2.75±0.46 A	1.41±0.41	$F_{(4,55)}$= 3.607, p<0.05
	0.66±0.30	1.08±0.35	1.33±0.44 A,B	$F_{(4,55)}$= 2.230, p=0.078
	*	*	ns	
	$F_{(1,22)}$=10.156, p<0.01	$F_{(1,22)}$=8.118, p<0.01		
	12.3±4.11	6.5±1.22	1.6±1.23	
	12.9±4.44	15.3±3.88	1.4±0.4345	
	ns	* $F_{(1,22)}$=11.642, p<0.01	A,B,C,D ns	$F_{(4,115)}$=4.476, p<0.01
	5.5±1.17	2.1±0.62 A,B,C	1.1±0.56 A,B,C	$F_{(4,55)}$= 8.194; p<0.001
	6.0±1.22	7.16±1.61	2.9±0.88	$F_{(4,55)}$= 1.128; n.s.
	ns	*	ns	
		$F_{(1,22)}$=8.305, p<0.01		
	504.4±178.2	196.3±68.59	215.3±133.4	
	1411±169.8	1303±186.2	1004±219.3	
	*	*	*	
	$F_{(1,22)}$=13.568, p<0.01	$F_{(1,22)}$=31.127, p<0.001	$F_{(1,22)}$=9.429, p<0.01	$F_{(4,115)}$= 1.293; n.s.

Table 4: Continued

Dose of F-13714, mg/kg	0 mg/kg **A**	0.0025 mg/kg **B**
Parameters measured	Mean ±SEM	Mean ±SEM
Latency 1st M (s) SERT +/+	155.5±71.6	44.17±19.21
SERT -/-	619.6±251.7	512.1±227.0
One-way ANOVA between genotype per dose	* $F_{(1,22)}$=3.144, p<0.05	**ns**
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=3.160, p<0.05 No Dose*Genotype effect $F_{(4,88)}$=1.79, n.s. Genotype effect $F_{(1,22)}$=22.47, p<0.001	
Latency 1st I (s)		
SERT +/+	302.6±152.4	120.4±50.65
SERT -/-	770.1±262.5	722.86±211.3
One-way ANOVA between genotype per dose	**ns**	* $F_{(1,22)}$=7.682, p<0.05
2-WAY ANOVA repeated measures	Dose effect $F_{(4,88)}$=4.628, p<0.001 Dose*Genotype effect $F_{(4,88)}$=2.298, p<0.05 No Genotype effect $F_{(1,22)}$=1.029, n.s.	
IR1 SERT +/+	35.5±4.4	45.3±4.47
SERT -/-	16.8±5.1	17.3±5.19
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(4,88)}$=1.514, n.s. No Dose*Genotype effect $F_{(4,88)}$=1.291, n.s. No Genotype effect $F_{(1,22)}$=1.029, n.s.	

A: significant difference compared to saline group, B: significant difference compared to 0.0025mg/kg group, C: significant difference compared to 0.01/mg/kg group;

	0.01 mg/kg C	0.04 mg/kg D	0.16 mg/kg E	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	Mean ±SEM	
	170.8±148.3 296.4±182.36 **ns**	369.0±195.7 327.2±198.8 **ns**	858.3±221.2 635.5±221.2 **ns**	$F(_{4,115})$= 2.315, n.s.
	201.8±146.0 319.7±181.0 **ns**	472.2±192.2 342.6±197.0 **ns**	945.9±209.2 **A,B,C,D** 832.5±234.0 **ns**	$F(_{4,115})$= 4.203, p<0.01 $F(_{4,115})$= 1.266, n.s.
	42.2±8.22 34.4±5.47 **NA**	49.8±10.64 34.1±8.11 **NA**	37.3±11.99 41.0±11.00 **NA**	**NA**

D: significant difference compared to 0.04/mg/kg group p values set at <0.05 (for specific p values go to results sections). *: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ (p<0.05).

Table 5: Effects of S15535 on Sexual Behavior of male SERT$^{+/+}$ and SERT$^{-/-}$ Wistar rats of batch one. N=12/group

Dose of S-15535 mg/kg	0 mg/kg	0.25 mg/kg
Parameters measured	Mean ±SEM	Mean ±SEM
# E SERT +/+	3.2±0.39	2.4±0.49
SERT -/-	1.1±0.32	0.9±0.28
One-way ANOVA between genotype per dose	* $F_{(1,22)}$=11.851, p<0.001	* $F_{(1,22)}$=6.776, p<0.001
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=0.616, n.s. No Dose*Genotype effect $F_{(4,3,66)}$=0.360, n.s. Genotype effect $F_{(1,22)}$=21.167, p<0.001	
# M 1st series		
SERT +/+	9.2±3.03	9.6±4.05
SERT -/-	14.1±5.12	19.0±3.99
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=0.439, n.s. No Dose*Genotype effect $F_{(3,66)}$=1.575, n.s. No Genotype effect $F_{(1,22)}$=4.45, n.s.	
# I 1st series		
SERT +/+	4.5±0.54	4.5±0.73
SERT -/-	7.0±1.8	7.9±1.20
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=0.827, n.s. No Dose*Genotype effect $F_{(3,66)}$=0.525, n.s. Genotype effect $F_{(1,22)}$=3.03, p<0.05	
Latency 1st E (s) SERT +/+		
SERT -/-	246.7±94.03 1122±185.5	602.6±213.0 1243±172.5
One-way ANOVA between genotype per dose	* $F_{(1,22)}$=11.051, p<0.001	* $F_{(1,22)}$=5.462, p<0.05
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=0.869, n.s. No Dose*Genotype effect $F_{(3,66)}$=0.346, n.s. Genotype effect $F_{(1,22)}$=25.627, p<0.001	

	1 mg/kg	.4 mg/kg	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	
	2.9±0.45	2.5±0.41	
	1.0±0.32	1.0±0.31	
	*	*	NA
	$F_{(1,22)}$=6.069, p<0.05	$F_{(1,22)}$=11.380, p<0.001	
	7.5±1.65	17.4±5.86	
	23.1±5.43	15.1±3.92	
	NA	NA	NA
	5.5±0.97	5.9±1.14	
	7.6±1.56	7.0±1.24	
	NA	NA	NA
	523.7±175.8	592.5±213.1	
	1288±148.1	1153±170.3	
	*	*	NA
	$F_{(1,22)}$=4.218, p<0.05	$F_{(1,22)}$=17.732, p<0.001	

Table 5: Continued

Dose of S-15535 mg/kg	0 mg/kg	0.25 mg/kg
Parameters measured	Mean ±SEM	Mean ±SEM
Latency 1st M (s) SERT +/+		
SERT -/-	18.4±6.85	73.5±38.28
	328.3±198.6	192.3±147.0
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=0.982, n.s. No Dose*Genotype effect $F_{(3,66)}$=0.988, n.s. No Genotype effect $F_{(1,22)}$=1.864, n.s.	
Latency 1st I (s)		
SERT +/+	63.5±31.13	254.2±153.3
SERT -/-	507.3±226.0	278.8±155.2
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=1.308, n.s. No Dose*Genotype effect $F_{(3,66)}$=1.008, n.s. No Genotype effect $F_{(1,22)}$=1.674, n.s.	
IR_1 SERT +/+	44.4±6.19	38.7±5.20
SERT -/-	28.2±6.39	30.6±5.16
One-way ANOVA between genotype per dose	* $F_{(1,22)}$=3.440, p<0.05	**ns**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,66)}$=0.375, n.s. No Dose*Genotype effect $F_{(3,66)}$=0.228, n.s. Genotype effect $F_{(1,22)}$=6.648, p<0.05	

*: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ (p<0.05).

	1 mg/kg	.4 mg/kg	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	
	35.2±23.27 176.3±147.8	11.2±3.13 99.5±55.39	
	NA	**NA**	**NA**
	328.3±198.8 477.7±210.0	33.1±8.30 280.6±147.4	
	NA	**NA**	**NA**
	40.3±7.51 23.5±5.08	44.2±8.0 31.6±6.3	
	ns	*	**NA**
		$F(_{1,22})$=3.294, p<0.05	

Table 6: Effects of S15535 on Sexual Behavior of male SERT$^{+/+}$ and SERT$^{-/-}$ Wistar rats of batch two. N=10 and N=11 respectively

Dose of S-15535 mg/kg	0 mg/kg A	0.25 mg/kg B
Parameters measured	Mean ±SEM	Mean ±SEM
# E SERT +/+	0.70±0.39	1.00±0.47
SERT -/-	0.54±0.24	0.54±0.31
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,57)}$=0.582, n.s. No Dose*Genotype effect $F_{(3,57)}$=0.524, n.s. No Genotype effect $F_{(1,19)}$=0.031, n.s.	
# M SERT +/+	19.20±1.34	10.70±3.11
SERT -/-	17.45±2.836	14.18±2.67
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	Dose effect $F_{(3,57)}$=3.161, p<0.05 No Dose*Genotype effect $F_{(3,57)}$=0.393, n.s. No Genotype effect $F_{(1,19)}$=0.002, n.s.	
# I SERT +/+	14.30±3.66	7.70±2.20
SERT -/-	12.27±2.12	8.36±2.05
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,57)}$=1.751, n.s. No Dose*Genotype effect $F_{(3,57)}$=0.293, n.s. No Genotype effect $F_{(1,19)}$=0.101, n.s.	
Latency 1st E (s) SERT +/+		
SERT -/-	1458±171.3	1324±203.9
	1490±140.6	1440±189.6
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,57)}$=2.049, n.s. No Dose*Genotype effect $F_{(3,57)}$=0.436, n.s. No Genotype effect $F_{(1,19)}$=0.011, n.s.	
Latency 1st M (s) SERT +/+		
SERT -/-	52.00±15.96	209.5±176.8
	48.16±11.60	98.48±52.75
One-way ANOVA between genotype per dose	**NA**	**NA**

	1 mg/kg C	.4 mg/kg D	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	
	0.90±0.31 0.90±0.25 NA	1.00±0.39 1.36±0.45 NA	NA
	20.00±4.03 17.82±2.40 NA	12.20±3.60 12.18±3.43 A,C NA	$F(_{3,80})$=2.946, p<0.05
	12.50±1.56 13.55±2.15 NA	8.70±3.01 11.64±4.04 NA	NA
	1324±168.4 1256±149.6 NA	1254±192.8 1194±196 NA	NA
	56.67±26.87 24.32±5.28 NA	218.2±176.5 77.22±41.22 NA	NA

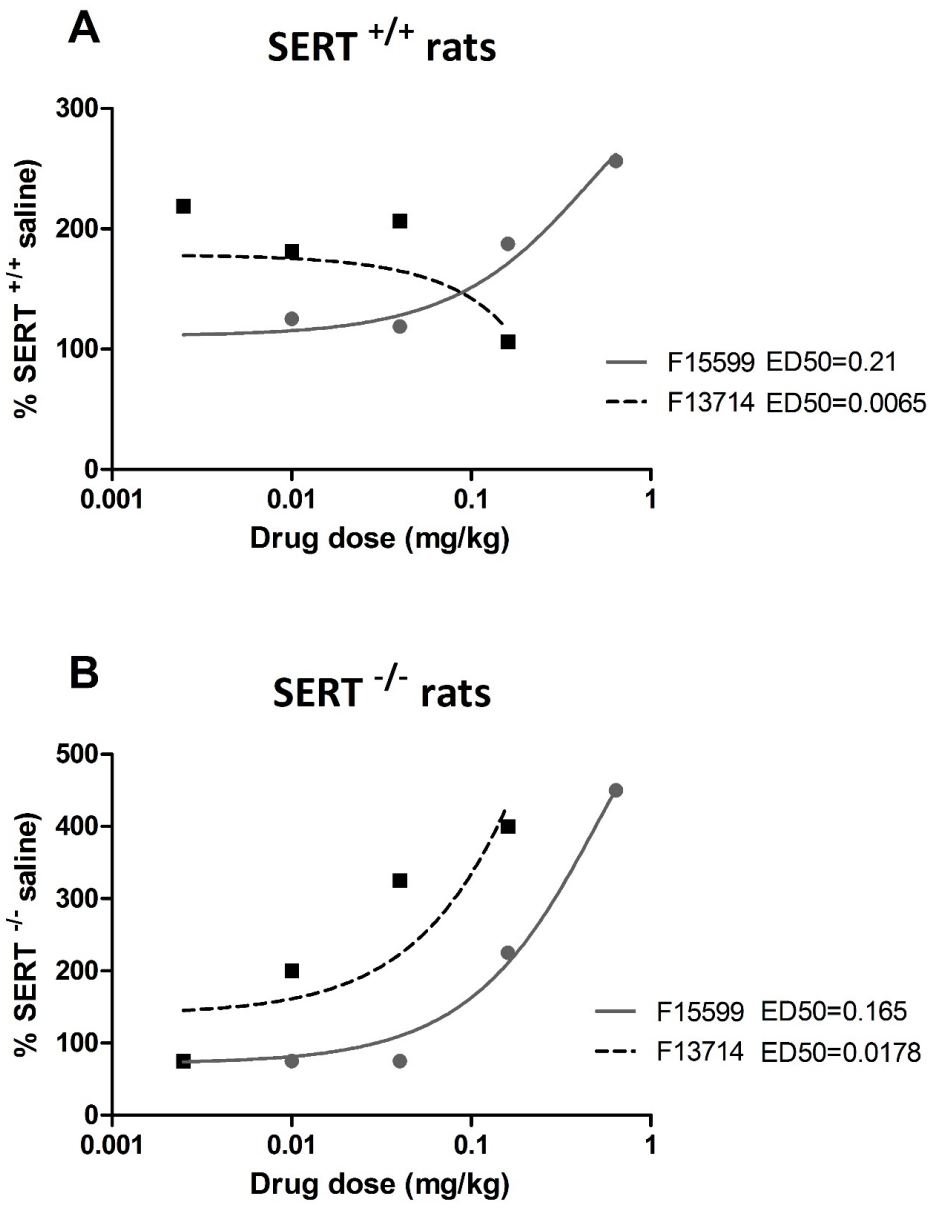
Table 6: Continued

Dose of S-15535 mg/kg	0 mg/kg **A**	0.25 mg/kg **B**
Parameters measured	Mean ±SEM	Mean ±SEM
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,57)}$=1.266, n.s. No Dose*Genotype effect $F_{(3,57)}$=1.373, n.s. No Genotype effect $F_{(1,19)}$=0.653, n.s	
Latency 1st I (s)		
SERT +/+	256.0±86.47	530.8±221.5
SERT -/-	82.19±10.46	567.9±240.0
One-way ANOVA between genotype per dose	* $F_{(1,19)}$=4.391, p<0.05	**ns**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,57)}$=1.266, n.s. No Dose*Genotype effect $F_{(3,57)}$=1.373, n.s. Genotype effect $F_{(1,19)}$=6.499, p<0.05	
IR_1 *SERT +/+*	322.1±27.51	367.7±32.40
SERT -/-	316.8±12.66	354.3±18.20
One-way ANOVA between genotype per dose	**NA**	**NA**
2-WAY ANOVA repeated measures	No Dose effect $F_{(3,57)}$=1.197, n.s. No Dose*Genotype effect $F_{(3,57)}$=0.564, n.s. No Genotype effect $F_{(1,19)}$=0.011, n.s	

A: significant difference compared to saline group, C: significant difference compared to 1/mg/kg group; p values set at <0.05 (for specific p values go to results sections). *: significant difference between $SERT^{+/+}$ and $SERT^{-/-}$ (p<0.05).

	1 mg/kg C	.4 mg/kg D	One Way ANOVA Dose effect
	Mean ±SEM	Mean ±SEM	
	77.90±31.63 97.57±52.92	517.3±223.0 537.6±244.7	NA
	ns	ns	
	401.3±13.31 461.5±93.61	357.0±15.43 375.6±35.51	NA
	NA	NA	

Supplementary figure



Fitted curve plot for SERT$^{+/+}$ (A) and SERT$^{-/-}$ rats (B). Data were normalized against the saline treatment and ED50 was calculated.